REMARKS

Amendments to the Specification

Applicants submit that the amendments to the specification merely correct the much regretted typographical errors with certain reduced isoalpha acids as isoadhumulones. Applicants respectfully submit that this typographical error is an obvious error because just as in dihydro-, tetrahydro-, and hexahydro-**iso**humulones or dihydro-, tetrahydro-, and hexahydro-**iso**cohumulones recited in the specification and the claims, "dihyro-, tetrahydro-, and hexahydro-adhumulone" must have been "dihydro-, tetrahydro- and hexahydro-**iso**adhumulone" to properly refer to the intended isomerized analogs of the hydrogenized **iso**alpha acids claimed. Support for the correct naming can be found, for example, in the structures provided in Figures 2 and 3C-3E. Entry of the these amendments is respectfully requested.

Amendments to the Claims

Claims 4 and 9-13 were previously pending and under examination. With this amendment, claims 4 and 9-13 have been cancelled without prejudice and new claims 14-30 have been added.

Support for claims 14-23 can be found in original claims and claims filed with a preliminary amendment in this case on June 18, 2007. Additional support for these claims can be found in Example 4 (with respect to the Combination Index feature); page 16 of the application as filed, paragraph 59, line 15; Figures 4A-4H; and previously presented claims 9-13. Support for claims 24-30 can be found in previously presented claims 4 and 9-13; the specification at page 16, paragraph 59, line 15; and Figures 4A-4H.

Entry of the above amendments and reconsideration in view the above amendments and the following remarks are respectfully requested.

1. CLAIM REJECTIONS UNDER 35 USC § 112

The Office has rejected claims 4, 10, and 11 under 35 USC § 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the specification was filed, had possession of the claimed invention. The Office contends that "the specification as originally filed does not provide support for the limitations "wherein the composition comprises from about 50 mg to about 7500 mg of the reduced isoalpha acid" and "wherein the composition comprises from about 50 mg to about 7500 mg of the isoalpha acid" for newly introduce claims 10 and 11 respectively. The original specification discloses that the "composition can be formulated to deliver about 50 to about 7500 mg of hops fraction, and not 50 to about 7500 mg of the reduced isoalpha acid, 50 to about 7500 mg of the isoalpha acid." Applicants respectfully disagree.

Claims 4 and 10-11 have been cancelled. As such, the ground for this rejection is now moot. Nonetheless, in response to this rejection and in view of the new claims 14-30, the Office's attention is directed to paragraph [0059] of the specification as filed and presented below where the highlighted section (bolded and underlined) provide literal support for the limitations "wherein the composition comprises from about 50 mg to about 7500 mg of the reduced isoalpha acid" and "wherein the composition comprises from about 50 mg to about 7500 mg of the isoalpha acid" for newly introduce claims 10 and 11 respectively.

> [0059] The invention provides methods that include delivering an effective amount of hops fractions, hops compounds, or hops derivatives alone or in combination with an additional active ingredient, as disclosed herein. For example, a daily dose of compositions of the invention can be formulated to deliver about 0.5 to about 10,000 mg of a hops fraction, for example, alpha acid, isoalpha acid, reduced isoalpha acid, tetra-hydroisoalpha acid, hexa-hydroisoalpha acid, beta acid, spent hops, or other hops fractions, per day. **In particular, an effective daily dose of compositions can be formulated to deliver about 50 to about 7500 mg of hops fraction, for example, alpha acids, isoalpha acid, reduced isoalpha acid, tetra-hydroisoalpha acid, hexa-hydroisoalpha acid, beta acid, spent hops, or other hops fractions, per day.** For example, an effective daily dose of compositions can be formulated to deliver about 100 mg to about

> 5000 mg, about 200 mg to about 3000 mg, about 300 mg to about 2000 mg, about 500 to about 1000 mg of hops fraction per day. In one embodiment, the effective daily dose is administered once or twice a day. A certain embodiment provides a composition comprising about 0.5 to about 500 mg of isoalpha acid or reduced isoalpha acid, for example, about 50 to about 300 mg or about 100 to about 200 mg of isoalpha acid or reduced isoalpha acid per day. In another embodiment, the invention provides a composition comprising about 10 to about 3000 mg of reduced isoalpha acid, tetra-hydroisoalpha acid, or hexa-hydroisoalpha acid per day, for example, about 50 to about 2000 mg, about 100 to about 1000 mg, about 200 to about 750 mg, or about 250 to about 500 mg of reduced isoalpha acid, tetra-hydroisoalpha acid, or hexa-hydroisoalpha acid per day. Yet another certain embodiment provides a composition comprising about 50 to about 7500 mg of spent hops per day, for example, about 100 to about 6000 mg, about 200 to about 5000 mg, about 300 to about 3000 mg, about 500 to about 2000 mg, or about 1000 to about 1500 mg of spent hops per day. *(emphasis added)*

Accordingly, Applicants maintain that paragraph [0059] shows that they were in possession of the invention as claimed with respect to compositions containing from about 50 mg to about 7500 mg of the reduced isoalpha acids or isoalpha acids. Therefore, it is respectfully requested that this rejection be withdrawn.

II. CLAIM REJECTIONS UNDER 35 USC § 103(a)

Claims 4 and 4-13 stand rejected under 35 USC § 103(a) as being unpatentable over Kuhrts (US 2004/0137096, herein after "Kuhrts").

The Office contends that "Kuhrts teaches a pharmaceutical composition comprising hops extract consisting of iso-alpha acids (IAA), and reduced iso-alpha acids (RIAA) such as . . . dihydroiso-humulone, . . . and combinations thereof. It is also disclosed that iso-alpha acids which are combinations of reduced isoalpha acid (RIAA) and isoalpha acid (IAA) will be present in an amount of 0.05% to 10% by weight in the hops extract. . . ." Office Action, page 4. The Office acknowledges that "Kuhrts does not expressly teach the ratio of reduced isoalpha acid:isoalpha acid as about 3:1 to about 1:10, in the composition. Kuhrts does not expressly teach that the composition contains at least 0.1% of RIAA and IAA individually." Office Action, page 4. Nevertheless, the Office concludes that "[i]t would have been obvious to a

person of ordinary skill in the art at the time of invention to determine or optimize parameters such as effective amounts of the reduced isoalpha acid and isoalpha acid employed in the composition of Kuhrts, to obtain a desired effect such as reducing inflammation." Office Action, page 4. Applicants respectfully traverse.

Claims 4 and 10-13 have been cancelled. As such, the ground for this rejection is now moot. Nonetheless, in response to this rejection and in view of the new claims 14-30, Applicants submit that Kuhrts neither teaches nor suggests a therapeutic compositions consisting essentially of the enumerated RIAAs and IAAs with combination index (CI) of less than 1 for synergistic inhibition of PGE2 production or reduction of PGE2-mediated inflammation.

Throughout its specification, Khurts primarily teaches a composition of alpha acids and beta acids. See, for example, the abstract, paragraphs 27, 34 and the claims 1 and 43. Although Khurts, in paragraph 31, mentions in passing that "[c]ompositions are also described that consist primarily of the alpha acids in hops, with little or no beta acids," it present no enabling disclosure in support of that statement; nor does it teach a composition consisting essentially of the enumerated RIAAs and IAAs, presently claimed. In both Examples 1 and 2 in Khurts (paragraphs 34 and 43), the compositions taught include beta acids (i.e., lupulone, colupulone, adlupulone, prelupulone, and postlupulone, per paragraph 25 of Khurts) as required active agents. Therefore, a person of ordinary skill in the art familiar with the teachings of Khurts could not have predicted or would have had any reasonable expectation of success to prepare a composition consisting essentially of the RIAAs and IAAs, as presently claimed.

Moreover, from the Khurts's teachings, a person of ordinary skill in the art could not have predicted or would have had any reasonable expectation that a composition consisting essentially of the enumerated RIAAs and IAAs could possibly act synergistically to inhibit PGE2 production or reduce PGE2-mediated inflammation. Applicants submit that they have unexpectedly discovered that compositions of reduced isoalpha acids (i.e., dihydro isoalpha acids) and isoalpha acids, when combined in certain ratios and amounts, have synergistic anti-inflammatory effects. By teaching how combination index (CI) can be calculated (See Example 4, paragraph 100), Applicants have sufficiently taught how these certain ratios and amounts, where synergy is obtained for combinations of RIAAs and IAAs, can be calculated. See also the

highlighted areas in the tables in Figures 4A-4H, where CI is less than 1. As evidenced by the Chou et al. (J. Biol. Chem. 252:6438-6442 (1977), listed in the application as filed on page 30, paragraph 100; a copy of which is enclosed herewith), calculation of combination index takes into account the concentrations of the compounds being tested for synergy. Therefore, to one of ordinary skill in the art of drug development, a "combination index of less than one" is necessarily inclusive of amounts and ratios at which synergy is observed.

Indeed, by assessing the combination index for various combinations of RIAAs and IAAs, Applicants have shown that at different rations and amounts —correspond to CI of, for example, 1 or above 1—, combinations of RIAA and IAA will not only fail to act synergistically but also act antagonistically towards one another in inhibiting PGE2 production. This discovery is also unexpected and unobvious to one of ordinary skill in the art familiar with the teachings of Khurts. As such and because of the above reasons, Applicants respectfully submit that the present claims are novel and unobvious over Khurts. Withdrawal of this rejection is respectfully requested.

III. RE: CLAIM REJECTIONS UNDER 35 USC § 102(E) PER OFFICE ACTION MAILED 12/10/2009

In the Office Action previously mailed on 12/10/2009, the composition claims (then pending in the application) were rejected under 35 USC § 102(e) as being anticipated by Shahlal et al. (US 6,583,322, "Shahlal et al."). In that Office Action, the Office alleged that Shahlal et al. disclosed compositions comprising a reduced isoalpha acid (RIAA) and isoalpah acid (IAA) in "FIG.1; FIG.2; column 1, lines 14-24 and 60-63; and column 4, lines 2-25." The Office further alleged that "[i]t is disclosed that compositions therein which are mixtures of DHIA and IAA remained clear liquids at all ratios between about 1 and 99%, and comprise at least 0.1% of the composition. See column 18, lines 15-45." In view of claims 14-23 presented herein, Applicants respectfully traverse this rejection.

Applicants respectfully submit that the composition claims provided above are novel over the teachings of Shahlal et al. because of the reasons of record (see Applicants' previous response filed 10/28/2009) and because Shahlal et al. failed to teach a composition consisting

essentially of the enumerated RIAAs and IAAs. All Shahlal et al. disclosed was a mere statement in col. 18, lines 35-37 of that reference that "[m]ixtures of the DHIA and THIA and/or IA were compatible and remained clear liquids at all ratios between aboiut 1 and 99%." However, this statement cannot make Shahlal et al. an anticipatory reference against the present claims because it requires a third active agent (i.e., THIA) to be present in the compositions of DHIA and IA. Furthermore, for the same reasons provided above in response to the obviousness rejection, the present invention as claims is also unobvious over Shahlal et al.

IV. CONCLUSION

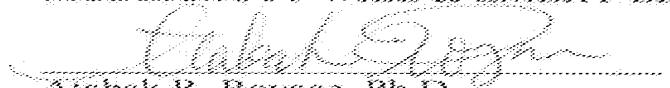
On the basis of the foregoing remarks and amendments, Applicants respectfully submit that the claims provided above are in condition for allowance. Passage to issue is respectfully requested.

If there are any outstanding issues that might be resolved by an interview or an Examiner's amendment, The Examiner is requested to call Applicants' agent at the telephone number shown below.

A Request for a Three (3) Month Extension of Time, up to and including **February 22, 2011** is included herewith insofar as the due date, February 19, 2010 is a Saturday and Monday February 21, 2011 is a federal holiday (President's Day). Pursuant to 37 C.F.R. § 1.136(a), the Examiner is authorized to charge any fee under 37 C.F.R. § 1.17 applicable in this instant, as well as in future communications, to Deposit Account 50-1133. Furthermore, such authorization should be treated in any concurrent or future reply requiring a petition for an extension of time under paragraph 1.136 for its timely submission, as constructively incorporating a petition for extension of time for the appropriate length of time pursuant 37 C.F.R. § 1.136(a) regardless of whether a separate petition is included.

Respectively submitted,

MCDERMOTT WILL & EMERY LLP



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A Simple Generalized Equation for the Analysis of Multiple Inhibitions of Michaelis-Menten Kinetic Systems*

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The summation of the effects of two or more reversible inhibitors of various types on the initial velocity of enzyme systems obeying Michaelis-Menten kinetics is described by the general relation:

$$\frac{1}{v_{i,j,\ldots,n}} = \sum_{i=1}^{n} \frac{1}{v_i} - \frac{n-1}{v_0}$$

wherein $v_{i,j,\ldots,n}$ is the velocity of reaction in the simultaneous presence of n inhibitors, v_i is the velocity observed in the presence of each individual inhibitor, and v_0 is the velocity in the absence of inhibition. The derivation is based on the assumption that each enzyme species can combine with no more than one of the inhibitors (i.e. the inhibitors are mutually exclusive). The above relationship holds irrespective of the number of inhibitors, the type of inhibition (competitive, noncompetitive, or uncompetitive), or the kinetic mechanism (sequential or ping-pong) of the enzyme reaction under consideration. Deviations from this equality define synergism or antagonism of inhibitors depending on whether the value of the left side of the above equation is greater or smaller than the right, respectively. Knowledge of the kinetic constants for substrates and inhibitors is not required. If two or more inhibitors act independently (i.e. are not mutually exclusive), their combined effects are necessarily synergistic. Under certain circumstances, described in the text, mutually nonexclusive inhibitors obey the fractional velocity product relationship:

$$v_{i,j,\ldots,n}/v_0 = (v_i/v_0) \times (v_j/v_0) \times (v_k/v_0) \ldots (v_n/v_0)$$

The present paper offers a novel, generalized, and exceptionally simple analysis of the effects of more than one inhibitor on the initial velocities of enzymatic reactions obeying Michaelis-Menten kinetics. We derive a relationship applicable to multiple, reversible, and mutually exclusive inhibitors, irrespective of their kinetic behavior (competitive, noncompetitive, or uncompetitive), and independent of the number of substrates involved, or whether the mechanisms are of the ordered (sequential) or of the ping-pong type. This rigorous definition of the summation of inhibitory effects makes possible the quantitative descriptions of synergism or antagonism among inhibitors.

Enzymatic reactions obeying Michaelis-Menten kinetics in the presence of varying concentrations of single inhibitors have been described in terms of three boundary conditions, in accordance with the effects of inhibitors on double reciprocal plots of initial reaction velocity with respect to substrate concentration (1, 2). Thus, the inhibitor may change the slope (competitive), the intercept on the ordinate (uncompetitive), or both (noncompetitive) of such graphs. In the case of single-substrate reactions, these conditions are the consequence of the binding of the inhibitor to free enzyme, E, only (competitive), to E and enzyme·substrate complex, EA, (noncompetitive), or to EA complex only (uncompetitive).[1] This report considers only pure boundary conditions and their permutations. Equations for mixed types of inhibitions can be derived similarly by introducing interaction factors (3, 4).

It is well known that for a given enzymatic reaction and inhibition mechanism, rate equations specific for each circumstance can be derived with steady state or rapid equilibrium analyses (3-6). Such rate equations always contain the maximum velocity term as well as the kinetic constants and concentration factors for each of the substrates and inhibitors. Algebraic rearrangement of these equations leads to useful alternative equations or graphical representations (7-13). We show herein that the algebraic rearrangement of these individual equations, and substitutions in each of them for multiple inhibitions, result in the cancellation of all kinetic constants, concentration parameters, and the maximum velocity term. An exceptionally simple general equation is thus obtained, which correlates the reaction rates in the presence of each inhibitor alone, with that observed in the simultaneous presence of all of these inhibitors. A preliminary account of this work has appeared (14).

There are several excellent experimental and theoretical studies of multiple inhibitions of individual enzymes (3, 4, 15-20). Many workers have made the simple assumption that the

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[1] In single-substrate reactions, uncompetitive inhibition is only a hypothetical situation. However, in many multisubstrate reactions, particularly those with ping-pong mechanisms, inhibition with respect to the secondary substrate is obligatorily uncompetitive.

effects of the simultaneous presence of two inhibitors can be predicted from the product of the fractional velocity observed in the presence of each inhibitor individually (3, 21). We show that this relationship is theoretically sound only under very restricted circumstances. Most of the earlier analyses have invoked the use of kinetic constants for substrates and inhibitors or have required the accumulation of extensive experimental measurements in order to obtain valid graphical representations. The derivations presented in this paper lead to quantitative descriptions of the summation of effects of multiple inhibitors of various types, require few measurements, and do not invoke the kinetic constants of substrates or inhibitors. Furthermore, our generalized relationships are readily applicable to the simultaneous action of more than two inhibitors. To our knowledge, Lienhard *et al.* (22) are the only workers who have recognized the possibility of such simple relationships. In the course of work on transition state inhibitors of ribonuclease, these authors (22) mention the relation $1/v_{i,j} = 1/v_i + 1/v_j - 1/v_o$ for a single-substrate reaction and two noninteracting inhibitors of competitive or noncompetitive type. However, the theoretical basis for this derivation and the range of its applicability were not developed.

NOMENCLATURE

The symbols and notations follow those proposed by Cleland (5):

v_o: initial velocity of uninhibited reaction

v_i, v_j, v_k ... v_n: initial velocity in the presence of inhibitors I_i, I_j, I_k ... I_n, respectively

$v_{i,j}$: initial velocity in the simultaneous presence of inhibitors I_i and I_j

$v_{i,j,k,...,n}$: initial velocity in the simultaneous presence of inhibitors I_i, I_j, I_k ... I_n

A,B: concentrations of substrates A and B, respectively

K_a, K_b: Michaelis constants for substrates A and B, respectively

K_i, K_j: inhibitor constants for inhibitors I_i and I_j, respectively

K_{ia}: dissociation constant for substrate A

I_i, I_j, I_k ... I_n: concentrations of inhibitors I_i, I_j, I_k ... I_n, respectively

V: maximum velocity of reaction

f_i: fractional velocity = v_i/v_o

$f_{i,j,k,...,n}$: fractional velocity in the simultaneous presence of n inhibitors

f_i': fractional inhibition = $(1 - f_i)$

ANALYSIS

Our initial analysis assumes that classical Michaelis-Menten kinetics is obeyed, that the inhibitors combine reversibly with the enzyme, and that each enzyme·inhibitor complex species contains only a single species of inhibitor, *i.e.* the inhibitors are mutually exclusive (4). We consider in turn, the relationships between the uninhibited initial velocities and those observed in the presence of one or more inhibitors, for reactions involving one or more than one substrate. Three specific cases are considered in the body of this paper, and further examples are developed in the miniprint supplement[*] (Appendix I).

Mutually Exclusive Inhibitors

Case 1.—One substrate reaction with two inhibitors: I_i is competitive, I_j is competitive.

$$v_o = VA/(K_a + A) \tag{1}$$

$$v_i = VA/K_a(1 + I_i/K_{ii}) + A] \tag{2}$$

$$v_j = VA/[K_a(1 + I_j/K_{ij}) + A] \tag{3}$$

$$v_{i,j} = VA/[K_a(1 + I_i/K_{ii} + I_j/K_{ij}) + A] \tag{4}$$

Combining Equations 1, 2, 3, and 4, hence:

$$1/v_{i,j} = 1/v_i + 1/v_j - 1/v_o \tag{5}$$

Case 2.—One substrate reaction with three inhibitors selected at random: I_i is competitive, I_j is noncompetitive, and I_k is uncompetitive.

$$v_i = VA/[K_a(1 + I_i/K_{ii}) + A(1 + I_i/K_{ia})] \tag{6}$$

$$v_k = VA/[K_a + A(1 + I_k/K_{ik})] \tag{7}$$

$$v_{i,j,k} = VA/[K_a(1 + I_i/K_{ii} + I_j/K_{ij}) + A(1 + I_i/K_{ia} + I_j/K_{ia})] \tag{8}$$

Combining Equations 1, 2, 6, 7, and 8, hence:

$$v_{i,j,k} = 1/v_i + 1/v_j + 1/v_k - 2/v_o \tag{9}$$

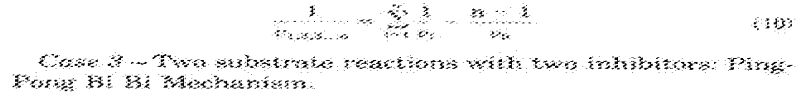
Extending the above arguments to four inhibitors of any classes, it may be seen that:

$$\begin{aligned} 1/v_{i,j,k,l} &= 1/v_i + 1/v_j + 1/v_k + 1/v_l - 3/v_o \\ &= 1/v_{i,j,k} + 1/v_l - 1/v_o \\ &= 1/v_{i,j} + 1/v_k + 1/v_l - 2/v_o \end{aligned}$$

Thus, the numerator of the reciprocal of the v_n term is equal to the number of partitions (the preceding terms) minus one. More generally, the velocities of single-substrate reactions in the presence of n inhibitors belonging to any combination of competitive, noncompetitive, and uncompetitive classes are expressed by the relation:[*]

$$\frac{1}{v_{i,j,k,...,n}} = \sum_{i=1}^{n} \frac{1}{v_i} - \frac{n-1}{v_o} \tag{10}$$

Case 3.—Two substrate reactions with two inhibitors: Ping-Pong Bi Bi Mechanism.



Two inhibitors: I_i is competitive with respect to substrate A (binds to E) and uncompetitive with respect to substrate B; I_j is competitive with respect to substrate A (binds to E) and uncompetitive with respect to substrate B.

$$1/v_o = [AB + K_aA + K_iK_b]/VAB \tag{11}$$

$$1/v_i = [AB + K_aA + K_iK_b(1 + I_i/K_{ii})]/VAB \tag{12}$$

$$1/v_j = [AB + K_aA + K_iK_b(1 + I_j/K_{ij})]/VAB \tag{13}$$

$$1/v_{i,j} = [AB + K_aA + K_iK_b(1 + I_i/K_{ii} + I_j/K_{ij})]/VAB \tag{14}$$

Combining Equations 11, 12, 13, and 14, again gives Equation 5, *i.e.*

$$1/v_{i,j} = 1/v_i + 1/v_j - 1/v_o \tag{5}$$

It is shown in the supplement that the general relation

[*] Alternative forms of this relationship, not involving the uninhibited velocity (v_o) term, are as follows:

$$f_{i,j,k,...,n} = 1 - \left[\sum_{i=1}^{n} (f_i)^{-1} - (n-1)\right]^{-1} \tag{10a}$$

$$= 1 - \left[1 + \sum_{i=1}^{n} (f_i'/f_i)\right]^{-1} \tag{10b}$$

The limit descriptor j is used here in place of i in order to avoid ambiguity. These alternative formulations are useful in analyzing inhibitory effects in which the uninhibited velocity is unknown (see Appendix V).

[*] Portions of this paper (including Appendixes I to V and Tables I to V) are presented in a miniprint following the references. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document 77M-165, cite authors, and include a check or money order for $3.40 per set of photocopies.

(Equation 10) holds for other combinations of inhibitors, and is equally applicable to ordered (sequential) as to ping-pong mechanisms.

Mutually Nonexclusive Inhibitors and the Fractional Inhibition Concept

A useful method for expressing the degree of inhibition of a reaction is in terms of the fractional velocity (f_i) which is the ratio of the velocity in the presence (v_i) to that in the absence (v_0) of the inhibitor. Consequently, the fractional inhibition (f_i') is $(1 - f_i)$. Numerous authors have intuitively assumed that the fractional reaction velocity in the presence of two or more inhibitors may be expressed as the product of the fractional velocities observed in the presence of each of the inhibitors individually. Thus, Webb (see Ref. 3, pp. 507–508)[2] states without theoretical support that for two inhibitors acting independently

$$f_{i,j} = f_i \times f_j \tag{15}$$

It is assumed tacitly that this relation describes a summation of inhibitory effects, since Webb (3) further proposes that synergism and antagonism among inhibitors should be defined in terms of deviations from Equation 15.

Our own analysis does not support this supposition, as may be seen from the following. Equation 15 may be transformed as follows:

$$v_{i,j}/v_0 = (v_i/v_0) \times (v_j/v_0)$$

whence

$$v_{i,j} = v_i v_j / v_0 \tag{16}$$

The generalized relationship developed in this paper for reciprocal velocities for two inhibitors (Equation 10) may be transformed as follows:

$$v_{i,j} = (v_i v_j v_0)/(v_i v_0 + v_j v_0 - v_i v_j) \tag{17}$$

or

$$f_{i,j} = (f_i f_j)/[(f_i f_j) + (f_i - f_i f_j) + (f_j - f_i f_j)] = (f_i f_j)/[1 - (f_i' \times f_j')] \tag{18}$$

Clearly Equations 15 and 18 are not identical.

The inhibited velocities calculated from the product of fractional velocities (Equation 15 or 16) will always be smaller than those predicted by Equation 10. In the case of more than two inhibitors, the disagreement between values given by Equation 10, and those calculated from the product of fractional velocities (Equations 15 or 16) becomes even larger. We conclude that if the assumptions of mutual exclusivity by reversible inhibitors obeying Michaelis-Menten kinetics apply, the analysis of multiple inhibitions by the product of fractional velocities (Equations 15 or 16) will always indicate synergism of inhibition (in comparison to the results predicted by Equation 10 for summation of inhibitory effects). The magnitudes of these discrepancies are illustrated in the supplement (Appendix II). However, it is shown in the supplement (Appendix III) that the product of fractional velocities accurately describes the behavior of two nonexclusive inhibitors provided at least one of these inhibitors is noncompetitive.

GENERALIZATIONS

Equation 10 describes the initial velocities of enzymatic reactions in the presence of multiple exclusive inhibitors. This relationship is independent of the number of substrates, the reaction mechanism, and the types or mechanisms of inhibitors. Consequently, we propose the following definitions of the effects of two inhibitors acting on a single target enzyme under steady state conditions:

Summation:

$$1/v_{i,j} = 1/v_i + 1/v_j - 1/v_0 \tag{5}$$

Synergism:

$$1/v_{i,j} > 1/v_i + 1/v_j - 1/v_0$$

Antagonism:

$$1/v_{i,j} < 1/v_i + 1/v_j - 1/v_0$$

By analogy, these relationships may be extended to larger numbers of inhibitors.

For mutually nonexclusive inhibitors, synergism will be invariably observed. Moreover, for noncompetitive, nonexclusive inhibitors, the relationship between inhibited and uninhibited velocities is given by the product of the respective fractional velocities.

$$v_{i,j,\ldots,n}/v_0 = (v_i/v_0) \times (v_j/v_0) \times (v_k/v_0) \ldots (v_n/v_0)$$

$$= \prod_{i=1}^{n} (v_i/v_0)$$

$$f_{i,j,\ldots,n} = f_i f_j f_k \ldots f_n = \prod_{i=1}^{n} f_i$$

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[2] Webb (3) uses the terms fractional activity (a) and fractional inhibition (i), where $i = (1 - a)$, and assumes that $a_{i,j} = a_i \times a_j$, which is identical with Equation 15.